Remarks

Claims 1, 6, 7, 10, and 14-21 are pending. The specification has been amended to replace "PHB polymerase" with "PHA synthase", as required by the Examiner. No new matter has been added.

Rejection Under 35 U.S.C. § 112, written description

Claims 1, 6, 7, 10, and 14-21 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection.

With regard to the NEW MATTER rejection, the Examiner alleges that there is no support for the amendment to claim 1, filed February 3, 2004, which recites "a *phbC* polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyrl CoA <u>and 3-hydroxyhexanoyl-CoA</u>". However, the Examiner's attention is directed to page 21, lines 11-15, which discloses that **3-hydroxyhexanoyl CoA accepting** PHA polymerase genes can be obtained from *A. caviae, C. testosteroni, T. pfenigii,* and possibly *P. denitrificans* and *S. natans*.

In addition, support can also be found in Example 3, which describes the production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) by construction of transgenic *E. coli* strains that express chromosomally encoded phaC from *N. salmonicolor*. Note that the only polymerase that is provided is the one obtained from *N. salmonicolor*, which acts on both substrates. Furthermore, Examples 2 and 5 disclose that the polymerase from *A. caviae* can **form** a PHBH copolymer, which demonstrates that enzymes that accept both 3-hydroxybutyrl

CoA and 3-hydroxyhexanoyl-CoA as substrates are not mere conjecture, but substantiated. The **sequence** for the *A. caviae* polymerase gene is disclosed in the publication cited in Example 2 on page 21, lines 24-26 (Fukui & Doi. *J. Bacteriol.* 179:4821-4830 (1997)). Finally, Figure 9 is a schematic of selection for a PHBH recombinant pathway in *E. Coli* using the PHA polymerase gene phaC from P. putida. The schematic clearly shows that the polymerase acts on 3-hydroxyhexanoyl-CoA. Accordingly, there is ample support in the specification for the amendments to claim and it is clear that the Applicants were in possession of the broad substrate polymerase at the time of filing.

With regard to the other claim rejections under 35 U.S.C. § 112, first paragraph (written description), please consider the following comments.

Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification. See e.g. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986). Applicants are claiming a method for the production of a polymer in bacteria that incorporates **new combinations** of genes and enzymes with **known** sequences.

The enzymes set forth in the claims are a phbA thiolase gene, a phbB reductase gene, a phbC polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl-CoA (claim 1), a gene encoding a ß-hydroxyacyl-ACP-coenzyme A transferase (claim 10), a gene encoding a D-specific enoyl-CoA hydratase (claim16), three enzymes from *C. acetobutylicum* that form butyryl CoA, a thiolase specific for 3-ketohexanoyl

CoA, a reductase specific for 3-ketohexanoyl CoA (claim 17), and fatty acid biosynthetic enzymes including ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase (claim 20). Each of these genes and enzymes were well known to those skilled in the art, commercially available and sufficiently identified in the specification as of the date of filing to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention.

For example, sequence information can be obtained from the cited publications, such as those on page 1, line 29 to page 2, lines 12, and actual DNA can be obtained from the authors of the cited publications or purchased from commercial suppliers, such as the American Type Culture Collection (ATCC). Published amino acid and nucleotide sequence listings for the various genes can also be obtained from GenBank or the National Center for Biotechnology Information (NCBI), as demonstrated by the Applicants in their response and amendment mailed on March 10, 2003, in which the Applicants submitted published sequence listings from the NCBI. Included were *phb*B amino acid and nucleotide sequences from *Asospirillum brasilense* and *Pseudomonas putida*; *phb*A amino acid and nucleotide sequences from *Pseudomonas putida*; and *phb*C amino acid and nucleotide sequences from *Pseudomonas putida*, *Rhodobacter sphaeroides*, *Azorhizobium caulinodans*, *A. eutrophus*, and *Pseudomonas sp*. In addition, in their response filed August 5, 2004, the Applicants submitted copies of the results of searches of GenBank for *acyl CoA synthase*, *acyl ACP thioesterase*, and *ACP-CoA transacylase* sequences published before the priority date of the application, January 22, 1998.

Furthermore, one of ordinary skill would know that each of the genes and enzymes used in the claimed methods can be easily isolated and sequenced using methods known in the art or

described in the specification. As noted above, on page 1, line 29 to page 2, line 11, the specification discloses a number of publications, which describe methods for producing PHAs in natural or genetically engineered organisms and makes special reference to patents that disclose the genes encoding reductase, thiolase, and PHB polymerase (U.S. Patent Nos. 5,245,023; 5,250,430; 5,480,794; 5,512,669; 5,534,432 to Peoples and Sinskey; page 2, lines 2-5). In addition, on page 10, line 29 to page 11, line 2, the specification states that useful PHA synthase genes have been isolated from, for example, *Aeromonas caviae* (Fukui & Doi, *J. Bacteriol.* 179: 4821-30 (1997)), *Rhodospirillum rubrum* (U.S. Patent No. 5,849,894), *Rhodococcus ruber* (Pieper & Steinbuechel, *FEMS Microbiol.Lett.* 96(1): 73-80 (1992)), and *Nocardia corallina* (Hall et. al., *Can. J. Microbiol.* 44: 687-91 (1998)). Furthermore, the Fukui & Doi and Hall references disclose that the PHA synthase genes from *A. caviae* (Fukui & Doi, page 4828, 1st full paragraph) and *Nocardia corallina* (Hall et al., pages 690-691) can polymerize 3-hydroxybutyrl CoA and 3-hydroxyhexanoyl-CoA.

The ß-hydroxyacyl-ACP-coenzyme A transferase gene encodes an enzyme that converts 3-hydroxyacyl ACP to the CoA derivative. This step in the polyhydroxyalkanoate pathway is facilitated by acyl ACP:CoA transferase activity. The specification states that genes that encode this enzyme can easily be identified in bacteria that produce polyhydroxyalkanoates from oxidized carbon sources, such as carbohydrates (see page 15, lines 18-23, of the specification). In addition, the identification of genes encoding enzymes that convert acyl ACP to acyl CoA is presented in Figure 10 as a screen that makes use of the very user friendly *lux* system of *Vibrio fischeri*. One merely assays for light generation that results from the induction of the transgenic

lux system. Such light generation implicates ACP::CoA transferase activity that is present in the system.

On page 24, lines 4-9, the specification recites a specific reference relating to the *pha*J gene encoding an enoyl-CoA hydratase (Fukui and Doi, *J. Bacteriol.* 179: 4821-30 (1997)), and describes how to isolate this gene from chromosomal DNA prepared from *A. caviae* strain FA-440, obtained from the Japanese Culture Collection under accession number FERM BP 3432 (U.S. Patent No. 5,292,860)).

On page 12, line 18 to page 13, line 19, the specification describes a thiolase specific for 3-ketohexanoyl, a reductase specific for 3-ketohexanoyl, and enzymes from *C. acetobutylicum* (*hbd, crt, bdh*) that form butyryl CoA. With regard to the enzymes from *C. acetobutylicum*, a number of publications are recited, which describe the isolation of these genes. In addition, Example 3 describes the isolation of the genes using PCR. The specification also makes reference to GenBank (page 13, line 4), demonstrating that the sequences of these genes may be accessed through a public depository. In addition, on page 11, lines 10-18, the specification recites Ploux *et al.* (1988) and Haywood et al.(1988), which disclose that 3-ketohexanoyl CoA is a substrate for reductase enzymes from *Z. ramigera* and *R. eutropha*. The specification also recites Haywood *et al.* (1988) on page 11, lines 19-24, which discloses that *R. eutropha* has two 3-ketothiolases, one of which has activity for higher 3-ketoacyl CoA's (i.e. 3-ketohexanoyl CoA).

Finally, the fatty acid biosynthetic enzymes are defined by their substrates. Many are known, cloned and well characterized. For example, see the enclosed copies of the results of

searches of GenBank for *acyl CoA synthase, acyl ACP thioesterase,* and *ACP-CoA transacylase* that were submitted by the Applicants in their response dated August 5, 2004. Homologous genes are readily isolated from bacteria such as *R. eutropha, A. latis, C. testosteroni, P. denitrificans, R. ruber*, and other PHA and non-PHA producers using the same methods to identify the *fao*AB (fatty acid oxidation) genes in *P. putida* KT2442. This is explicitly stated at lines 30-3, bridging pages 14 and 15 of the specification. Furthermore, epimerase activity had been detected in the fatty acid oxidation complexes of *E. coli* and *P. fragi*. As disclosed at page 14, lines 21-26, each of the *Fao*AB complex subunits were cloned and expressed to show the substrate specificity of components of the PHA pathway in *P. putida*.

Rejection Under 35 U.S.C. § 112, enablement

Claims 1, 6, 7, 10, and 14-21 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection.

The test of enablement is whether one of ordinary skill in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *United States v. Telectronics, Inc.*, 857 F.2d 778, 8 U.S.P.Q.2d 1217 (Fed. Cir. 1988); *In re Stephens,* 529 F.2d 1343, 199 U.S.P.Q. 659 (C.C.P.A. 1976). A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 U.S.P.Q.2d 13321, 1332 (Fed. Cir. 1991); *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 3 U.S.P.Q.2d 1737 (Fed. Cir. 1987).

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt,* 537 F.2d 498, 190 U.S.P.Q. 214

(C.C.P.A. 1976). Whether undue experimentation is needed is not based upon a single factor; it is a conclusion reached by weighing many factors. These factors have been summarized in *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988).

A proper analysis of the *Wands* factors shows that the claims satisfy the enablement requirement. The courts have indicated that some experimentation is permitted as long as such experimentation is not undue. As stated in *MIT v. A.B. Fortia*, "The fact that experimentation may be complex does not make it undue if the art typically engages in such experimentation". It clear from the amount of direction or guidance presented in the specification, the presence of working examples, the state of the prior art, and the relative skill in the art that one of ordinary skill in the art would be able to make and use the claimed transgenic bacteria for the production of polyhydroxybutyrate-co-polyhydroxyhexanoate without **undue experimentation.**

The issue is whether or not one skilled in the art would know what genes are required to make the claimed bacteria, and whether or not it would require undue experimentation to make and use the claimed bacteria. As demonstrated by the actual working examples in the specification, those skilled in the art would know what enzymes are required and would either be able to use those publicly available, described in the literature, or obtained without undue experimentation using the information provided by Applicants. Unlike in some systems, such as eukaryotic cells, genes have been identified by their activities and transferred into bacteria, either in plasmids or incorporated into the genome, without the sequence being known, for decades. What is important is to know a source for the genes, and what the genes must encode.

The specification clearly discloses how to obtain the genes and enzymes that are used in the methods and recites specific publications, which describe these materials in detail. For example, on page 1, line 29 to page 2, line 11, the specification discloses a number of publications, which describe methods for producing PHAs in natural or genetically engineered organisms. In addition, on page 10, line 29 to page 11, line 2, the specification discloses a number of organisms from which useful PHA synthase genes have been isolated. There is also adequate support in the specification for all of the claimed genes and enzymes, including a phbC polymerase gene encoding an enzyme that polymerizes 3-hydroxybutyryl CoA <u>and</u> 3-hydroxyhexanoyl CoA (page 21, lines 11-15 and Examples 2, 3 and 5). Furthermore, the genes and enzymes may be isolated using methods commonly known in the art or described in the publications, obtained from the authors of the cited publications, or purchased from commercial suppliers, such as the American Type Culture Collection (ATCC).

Once a gene is identified, it is routine in the art to incorporate the gene into a plasmid for transfection of bacteria. There is sufficient direction and guidance given by the specification to construct plasmids and express the claimed genes in bacteria (see page 18, lines 15-28 and Examples). Furthermore, the experimental protocols are routine in the art and expression vectors, restriction enzymes and ligation enzymes are also commerically available.

Although there is no requirement for examples, Applicants have provided numerous working examples which not only demonstrate that one can use the claimed enzymes to produce HH containing copolymers, but that one can isolate the desired enzymes with only routine experimentation. Example 1, on page 19 of the specification, discusses a routine method used

for the isolation of specific genes. This Example illustrates the amplification and isolation of the *pha*C gene, encoding the polyhydroxyalkanoate ("PHA") polymerase enzyme, from *N. salmonicolor* chromosomal DNA. Example 3 further shows, using the same methods of Example 1, how one would isolate the *hbd*, *crt*, and *bdh* genes from *C. acetobutylicum* (see page 22 of the specification). In addition, both Examples 1 and 3 describe cloning the isolated genes into plasmids.

The enzymes are defined by their substrate specificity. As discussed at page 5, lines 25-27, of the specification, "the genes are preferably selected on the basis of the substrate specificity of their encoded enzymes being beneficial for the production of the 3HH polymers." The substrate, in the presence of its cognate active enzyme, will be readily converted into product (i.e. the substrate for another enzyme). Based upon the specification, one of ordinary skill in the art will appreciate that the presence, or production, of end-product (i.e. polyhydroxybutyrate-co-polyhydroxyhexanoate) is easily measured and characterized using methods well known in the art.

Finally, Applicants remind the Examiner that they have patents on engineering of bacteria and plants to express PHA synthesis genes that issued on patent applications filed more than fifteen years ago. The massive amount of prior art clearly demonstrates that the field is not unpredictable, and that once one identifies the enzymes to be used, based on their known

substrates and known reaction products, it becomes routine to express the genes encoding those enzymes.

Allowance of claims 1, 6, 7, 10, and 14-21, is respectfully solicited.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: January 19, 2005

PABST PATENT GROUP, LLP
400 Colony Square, Suite 1200
1201 Peachtree Street
Atlanta, Georgia 30361
(404) 879-2151
(404) 879-2160 (fax)